Protocols for Reagent Delivery and Characterization of Flow Behavior in Microfluidic Chips

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Introduction

Increasingly broad applications for genetic analysis have led to a greater demand for improved DNA extraction techniques. Microfluidic technology provides an opportunity to extract and purify cellular DNA more quickly and cheaply than conventional procedures.[1,2] In this method, cells are delivered to channels within a microfluidic chip. These cells are then processed by a series of reagents that sequentially break apart the cell and expose long strands of DNA. The purified DNA is then removed for analysis.[3,4]

One challenge associated with microfluidic DNA extraction is the issue of reagent delivery (the "world-to-chip" problem).[5] Microfluidic channels are designed to hold nano- or microliters of reagents. However, typical reagent reservoirs hold milliliters or more. This project addresses the issue of scale by developing an effective protocol for dual-inlet reagent delivery to chips specifically designed for cellular DNA extraction.

Objectives

- Develop an effective protocol for simultaneously loading two reagents onto a microfluidic chip via dual inlet ports.
- Observe and qualitatively characterize flow through chip.

Microfluidic Chip Design

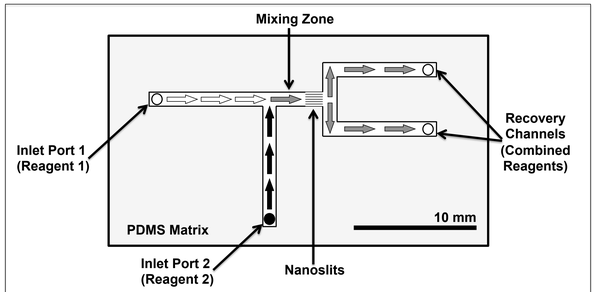

Figure 1 | Schematic design of dual-inlet PDMS microfluidic chip. Chip design by Paridhi Agrawal.

- Chips are made of polydimethylsiloxane (PDMS) bonded to a glass microscope slide.
- Chip dimensions: microfluidic channels are 0.3-0.9 mm wide and 30 µm deep; inlet channels are 10 mm long; mixing zones are 2.5-7.7 mm long; nanoslits are 25 µm wide and 500 nm deep.
- Reagents are transferred from off-chip fluid reservoirs to inlet ports 1 and 2 and then intersect in the mixing zone.

Materials and Methods: Testing Devices for Fluid Transfer

Transferring Fluid from Reservoirs to Chips:
- Two different devices (a Fluigent pressure controller and a Harvard Apparatus syringe pump) were used to transfer fluid to chips.
- The Fluigent controls pressure directly, using a range of 10-1000 millibars to create a pressure gradient. The syringe pump delivers reagents from syringes to chips by maintaining a constant volumetric flow rate.
- The Fluigent uses microcentrifuge tubes (1-2 mL capacity) as off-chip fluid reservoirs. Syringes (1-3 mL capacity) are the fluid reservoirs for the syringe pump. For both devices, connective tubing is used to attach the fluid reservoir to the microfluidic chip.

Fluid Delivery Procedure:
- Distilled water colored with food dye was used in place of of functional cell processing reagents.
- One reagent reservoir was connected to each chip inlet port. The Fluigent or syringe pump was then used to induce flow to the chip.
- For each test, the following data were recorded: pressure or flow rate required to induce flow; time for chip to fill (defined as time for fluid to reach recovery channels); and qualitative observations of backflow and fluid mixing.

Figure 2 | Devices used for fluid transfer.

Figure 2a | Fluigent pressure controller.

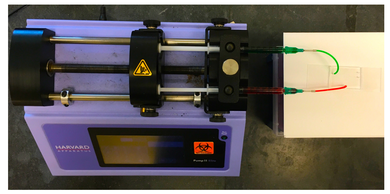

Figure 2b | Harvard Apparatus syringe pump.

Results and Discussion

Figure 3 | Simultaneous delivery of dyed distilled water to chip inlets 1 and 2 using syringe pump to control flow.

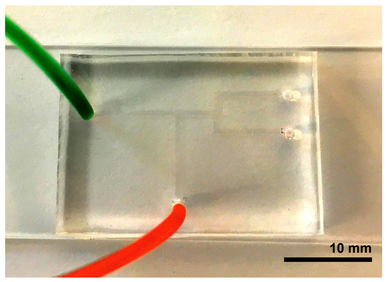

Figure 3a | Empty chip. At time t = 0 minutes, green and red streams are attached to inlet ports 1 and 2 respectively.

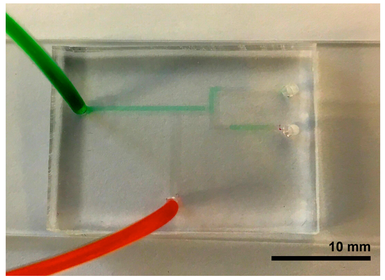

Figure 3b | Partially filled chip. At time t = 9 minutes, the green stream has moved through the mixing zone to the recovery channels.

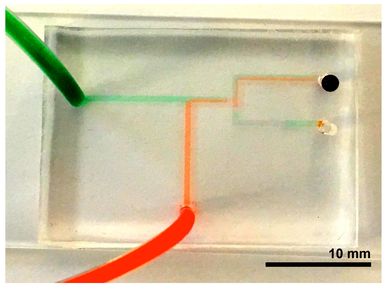

Figure 3c | Filled chip. At time t = 10 minutes, the red stream has entered the chip. The red and green streams flow side-by-side.

Fluigent Pressure Controller
- A range of pressures between 10-800 millibars was tested.
- Backflow was persistent: one stream would move through the other inlet channel instead of the mixing zone.
- No chips were successfully filled.

Harvard Apparatus Syringe Pump
- Dual-inlet reagent delivery was achieved.
- Timescale: using flow rates between 2-5 µL/min, it took approximately 7-15 minutes from the time fluid reached the inlets for the chip to fill.
- Some backflow was initially observed, but would eventually reverse, as one stream entered the chip before the other.

Observation: Unmixed Streams in Filled Chips
- Chips filled using the syringe pump exhibited lack of mixing between the streams from inlets 1 and 2.
- Because the microfluidic channel dimensions are so small, flow in the chip is laminar: liquid moves in parallel layers. Reagent mixing is a function of diffusion only.[6]
- For a channel width (w) of 0.6 mm, fluid velocity (v) of 2.7 mm/s (i.e., volumetric flow rate of 3 µL/min) and reagents with generic diffusivities (D) of approx. 10^{-9} m^2/s: the channel length required for mixing is approx. 0.25 m (from Péclet number).[7,8] Thus, complete mixing is unlikely.

Conclusions

Optimal Reagent Delivery Protocol:
- Use a syringe pump at low settings (2-5 µL/min) to control flow. Allow 7-15 minutes (from the time reagents reach the chip inlets) for the chip to fill with both reagents.

Limitations of Optimal Reagent Delivery Protocol:
- Uneven startup as one stream enters the chip first and is followed by the second stream could lead to uneven cell processing.
- Backflow from one reagent during startup delays entry of the other reagent, which increases processing time.
- Because fluid mixing depends on diffusion, the reagents require a channel much longer than the current mixing zone in order to fully mix.

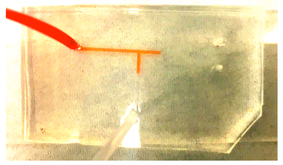

Figure 4 | Backflow in chip. Red stream from Inlet 1 has moved into the Inlet 2 entry channel.

Next Steps: Microbead Tests

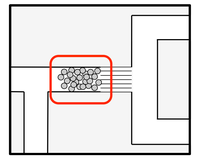

Figure 5 | Microbeads in mixing zone of chip.

Microbeads (spheres 5-15 µm in diameter) will be delivered to microfluidic chips to simulate cells. Reagents can be added to model the flow behavior in microfluidic channels containing cells.

Acknowledgements

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References

[1] Agrawal, P., Bognár, Z., and Dorfman, K. Entropic trap purification of long DNA. *Lab Chip* 2018, 18: 955-964.
[2] Benitez, J.J., et al. Microfluidic extraction, stretching and analysis of human chromosomal DNA from single cells. *Lab Chip* 2012, 12: 4848-4854.
[3] Rasmussen, K., et al. A device for extraction, manipulation and stretching of DNA from single human chromosomes. *Lab Chip* 2011, 11: 1431.
[4] Prinz, C., et al. Bacterial chromosome extraction and isolation. *Lab Chip* 2002, 2: 207-212.
[5] Liu, J.; Hansen, C; Quake, S.R. Solving the "World-to-Chip" Interface Problem with a Microfluidic Matrix. *Analytical Chemistry* 2003, 75: 4718-4723.
[6] Ward, K., and Fan, Z.H. Mixing in microfluidic devices and enhancement methods. *Journal of Micromechanical Engineering* 2016, 25.
[7] Lee, C-Y.; Chang, C-L.; Wang, Y-N.; and Fu, L-M. Microfluidic Mixing: A Review. *International Journal of Molecular Sciences* 2011, 12: 3263-3287.
[8] Mills, R. Self-Diffusion in Normal and Heavy Water in the Range 1-45°. *Journal of Physical Chemistry,* 1973, 77: 685-688.